

Figure 1

# Screening Protocol for Haptotactic-Migration Inhibitors: V 3.0

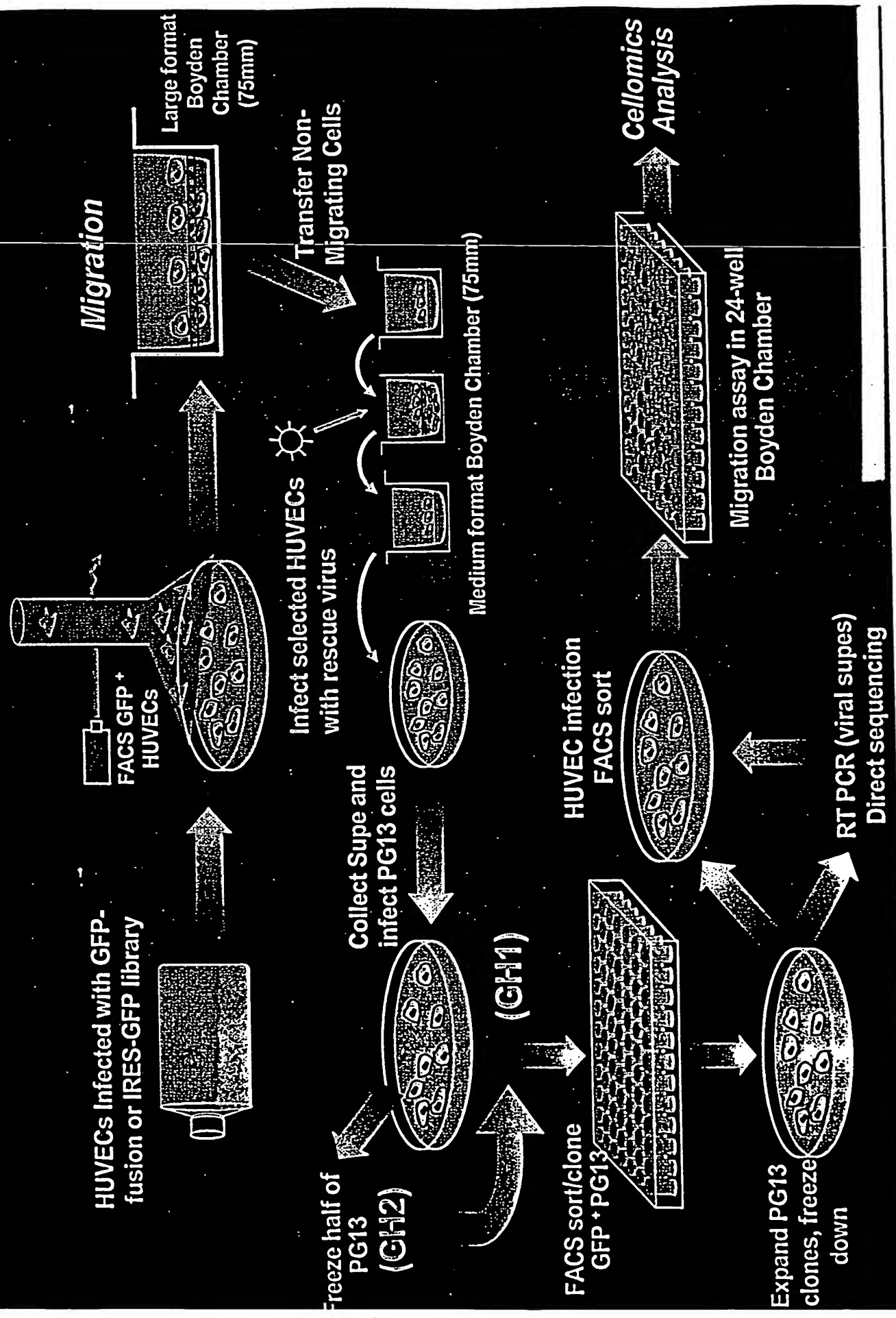


Figure 2

# Cellomics Haptotaxis Assay

Haptotaxis in Boyden chamber  
2000 cells / 24-well in triplicate  
0-15µg/ml vitronectin gradient for 3 hrs  
Fix and stain with DAPI

Count # cells on top / bottom  
using Cellomics image  
processing

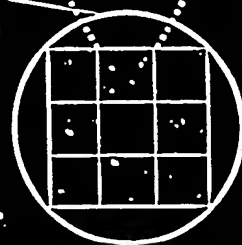
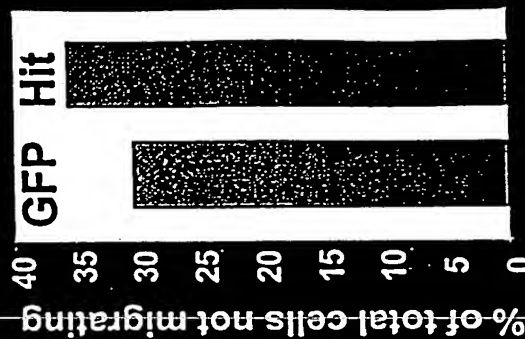
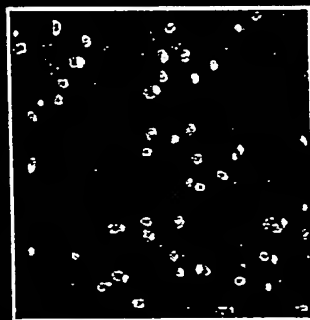
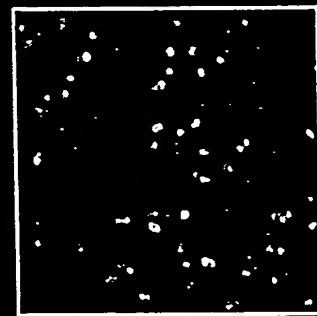


image 9 fields  
well



Data presented as  
% cells NOT  
migrating

- Cells directly imaged on membrane
- High accuracy in counting
- Distinguishes cells vs pores

Figure 3

# GFP-CD13 / N-aminopeptidase Screening Hit Inhibits Haptotaxis

TM

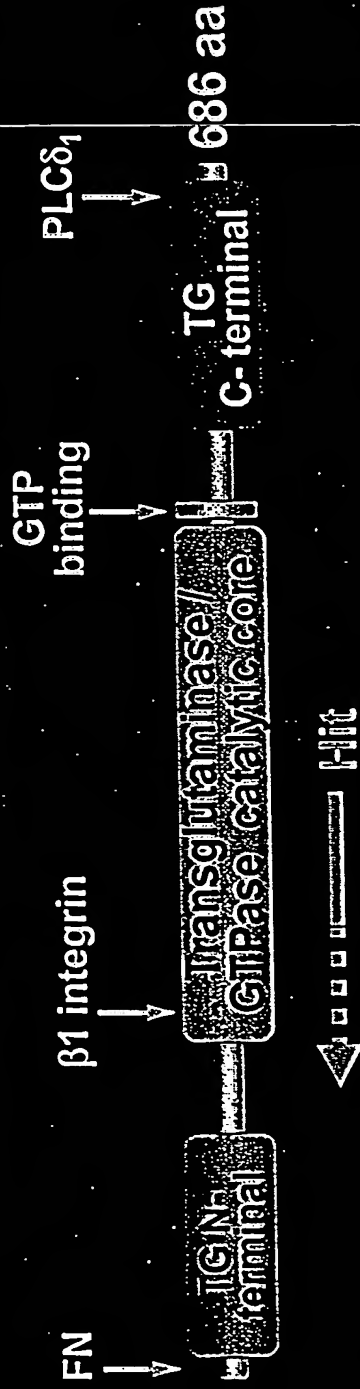


- Type II membrane protein
- M1 metalloprotease
- Expressed in myeloid cells and angiogenic endothelial cells; upregulated by VEGF and bFGF in HUVECS
- CD13 inhibitors impair tube formation, angiogenesis and tumor growth
- Expressed in RA synovia; T cell chemoattractant

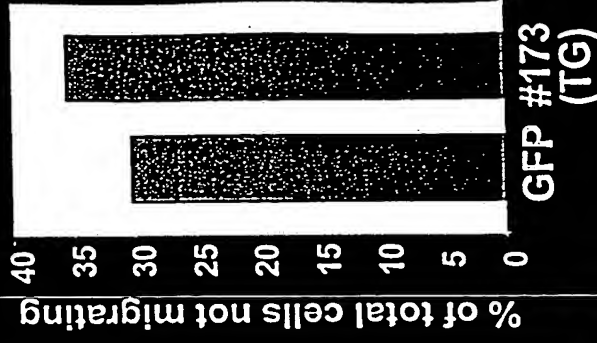


Figure 4

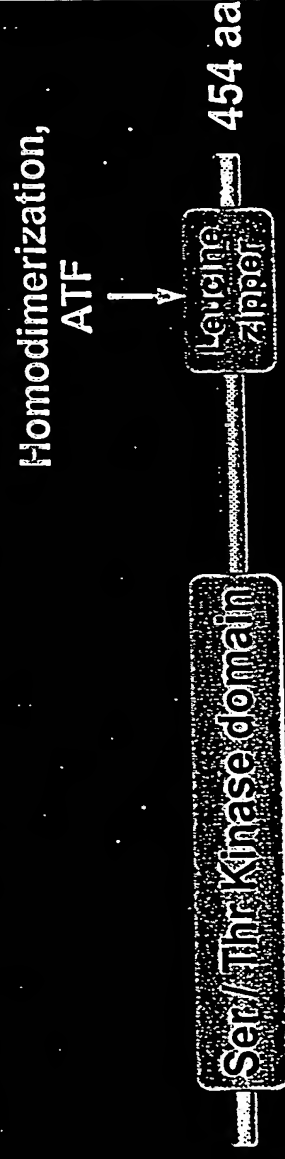
# GFP-Transglutaminase II Screening Hit Inhibits Haptotaxis



- Antisense hit
- Multifunctional protein
  - protein crosslinking
  - (de)amidation (e.g Rho)
  - GTPase mediates 7TM receptor signaling to PLCδ1
  - mediates integrin interactions
- Constitutively expressed at high levels in endothelial cells
- Expressed in endothelial cells during wound healing
- TG2 null mice exhibit impaired wound healing, autoimmunity and diabetes
- Recombinant transglutaminase promotes angiogenesis



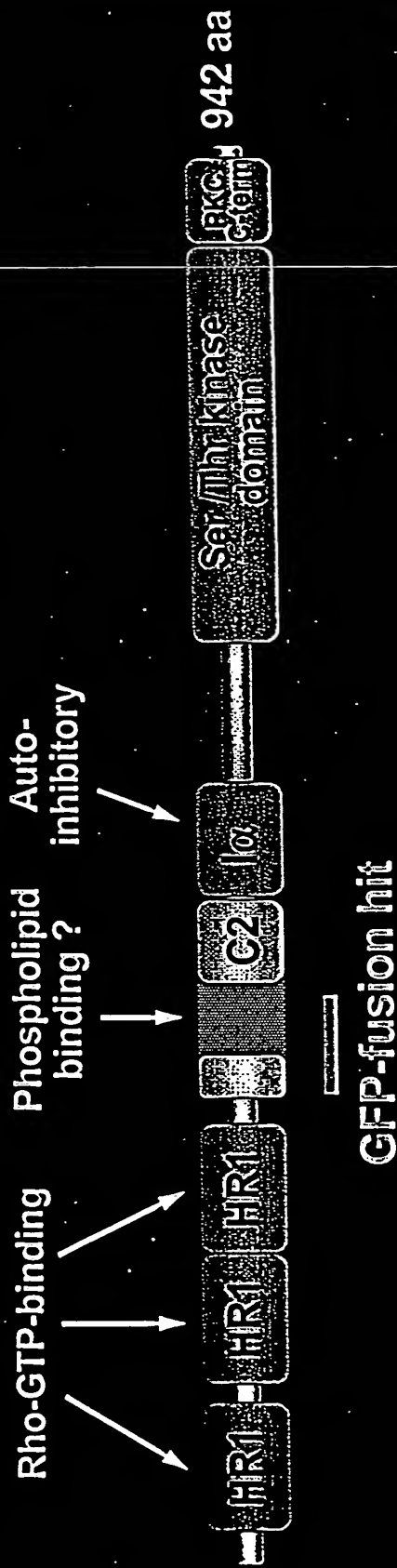
# GFP-Zip Kinase Screening Hit Inhibits Haptotaxis



- Hit is 3' untranslated sequence
- Overexpression causes apoptosis in NIH 3T3 cells, recombinant protein stimulates contraction of smooth muscle
- Phosphorylates MLC<sub>20</sub> in vitro, activating myosin ATPase activity and mediates Ca<sup>2+</sup>-independent SMC contraction
- Localized to nucleus (NIH 3T3), myofibrils (SMC) and actin filaments?

Figure 6

# GH1-54 Screening Hit Encodes a Fragment of PRK-1



- Cytoplasmic serine/threonine kinase related to PKC
- Hit GFP-fusion with part of C2 domain
- Binds Rho GTPase through N-terminus
- Regulated by activation loop phosphorylation by PDK1
- PRK activation by PDK1 is Rho-dependent and membrane-localized
- May regulate actin / myosin / microtubules

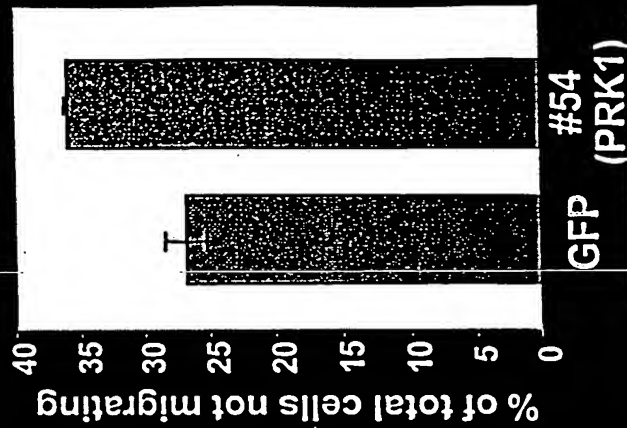


Figure 7

# PRK1 mRNA Expression is Restricted to Endothelial Cells and PBMCs

Taqman expression profile of primary cells (polyA+ RNA)

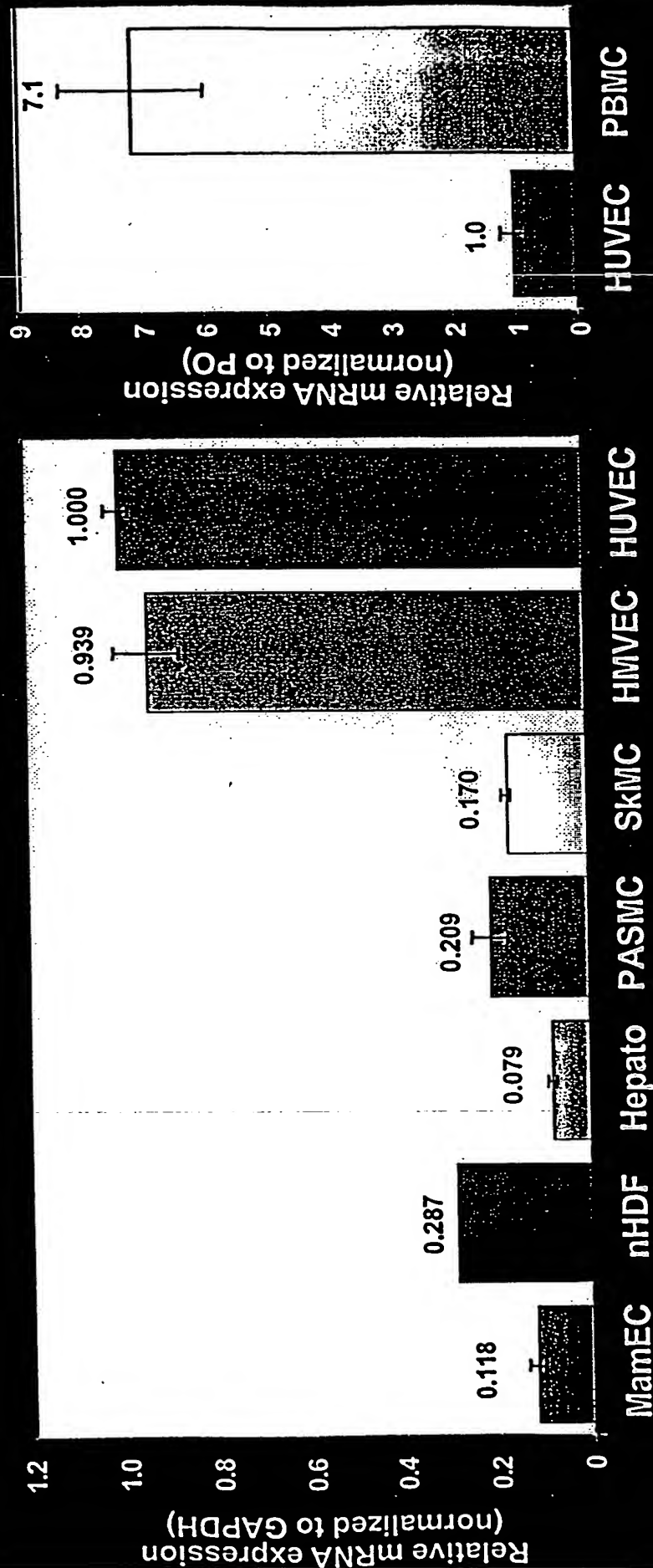


Figure 8

# GFP-PRK-1 Screening Hit Inhibits Haptotaxis and Reduces $\alpha\text{v}\beta 3$ Levels

Haptotaxis on  
Vitronectin Gradient

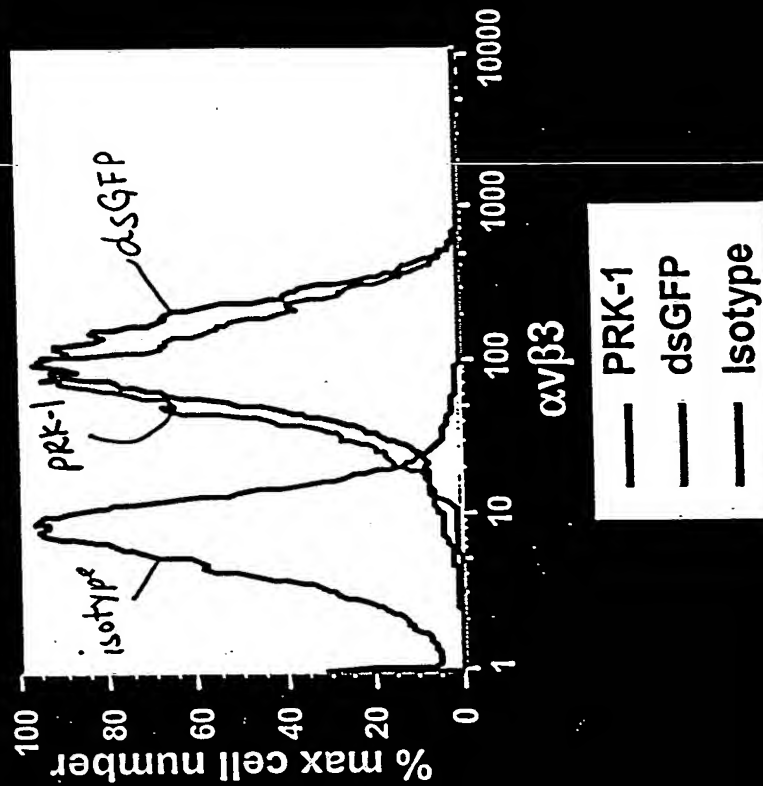
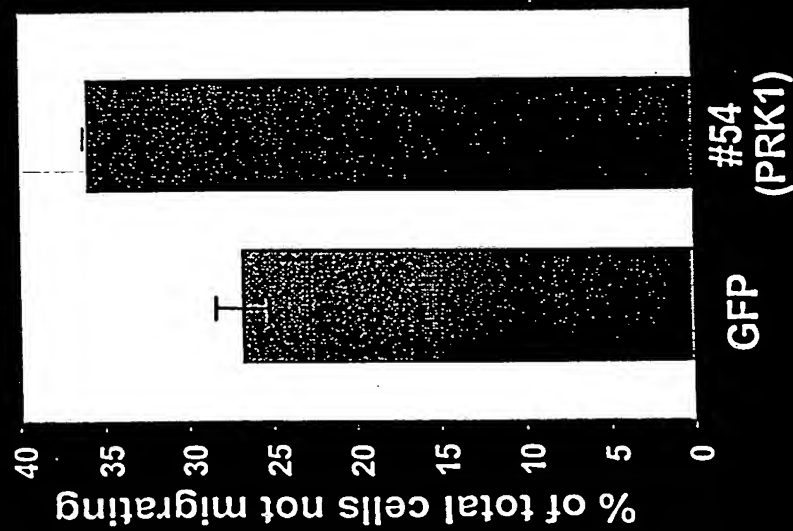
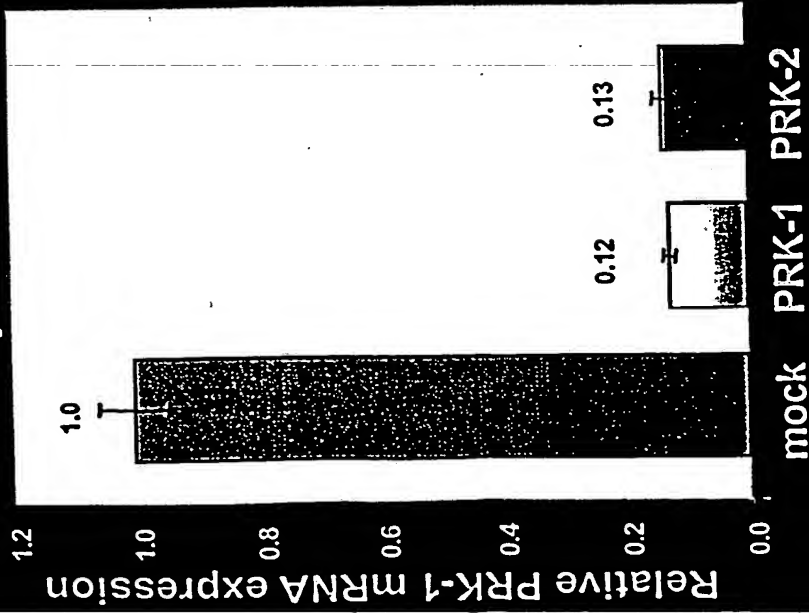




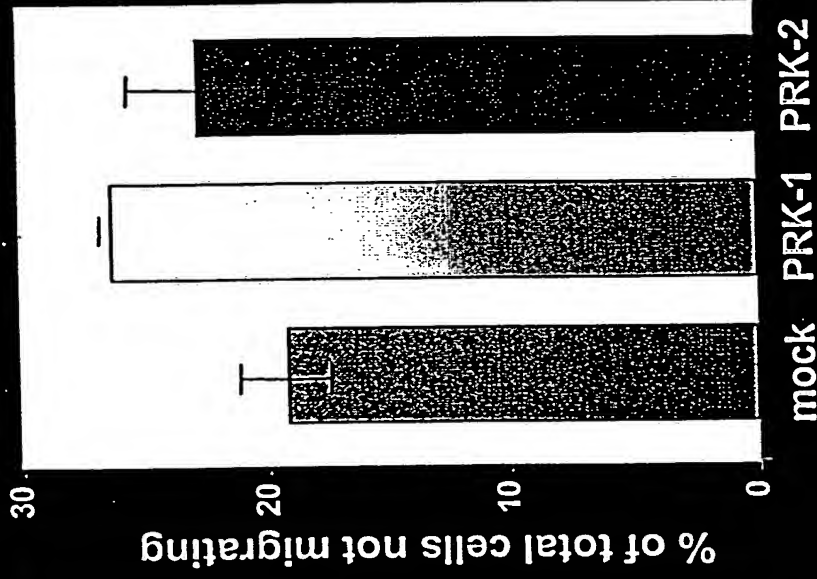
Figure 9

# PRK-1 RNAi Reduces PRK-1 Message, Haptotaxis and $\alpha v \beta 3$ Expression

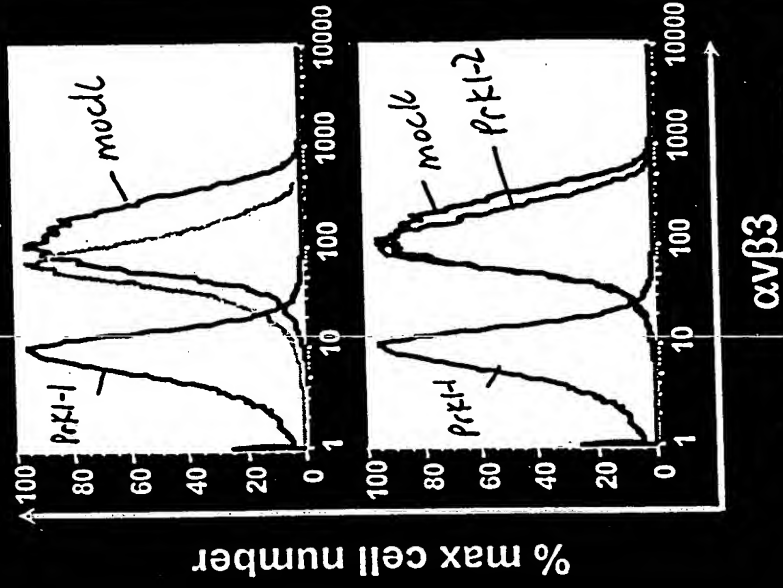
PRK-1 mRNA  
expression



Haptotaxis



$\alpha v \beta 3$



$\alpha v \beta 3$

PRK1-1  
PRK1-2  
mock

# PRK-1 RNAi Reduces Tube Formation in the Co-culture Assay

5 day co-culture stained with anti-CD31-FITC

Mock

PRK1-1

PRK1-2



# Axl and Gas6 were Isolated in the Haptotaxis Screen

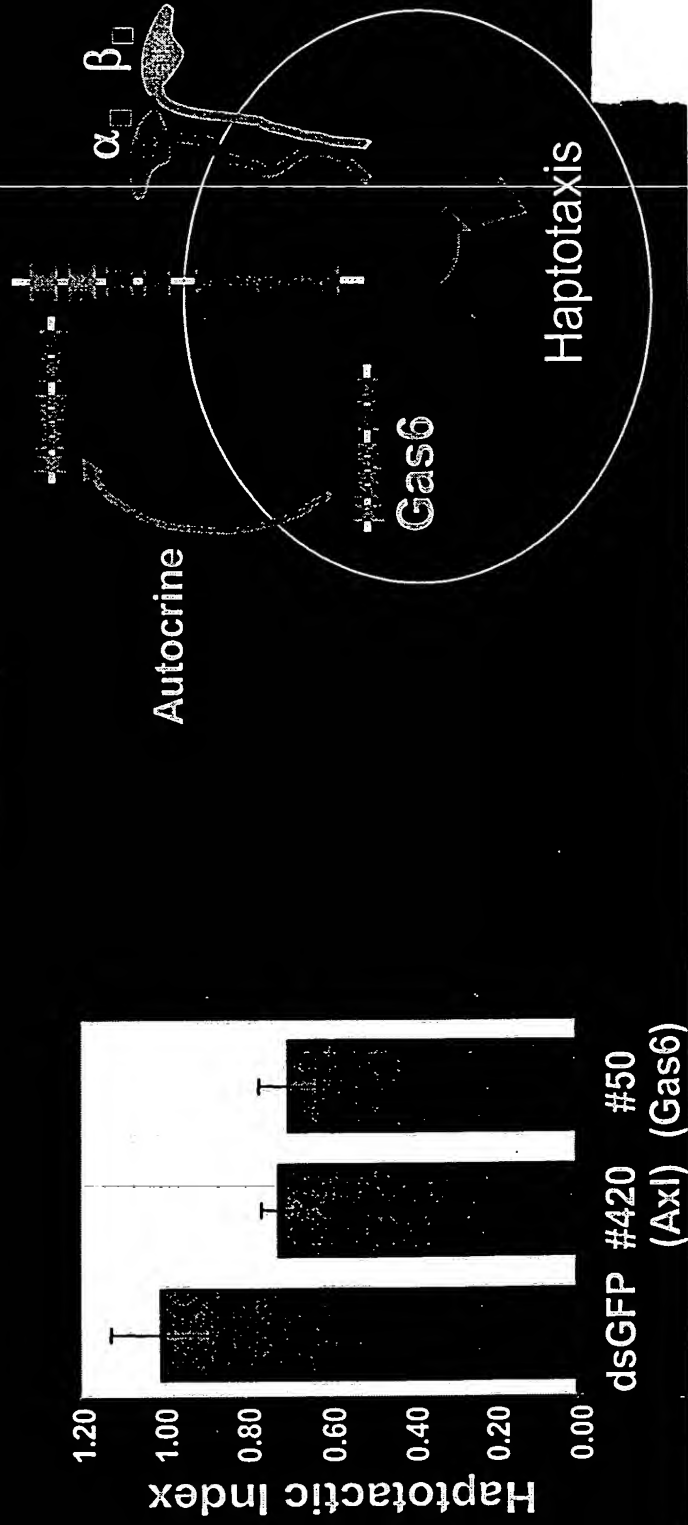
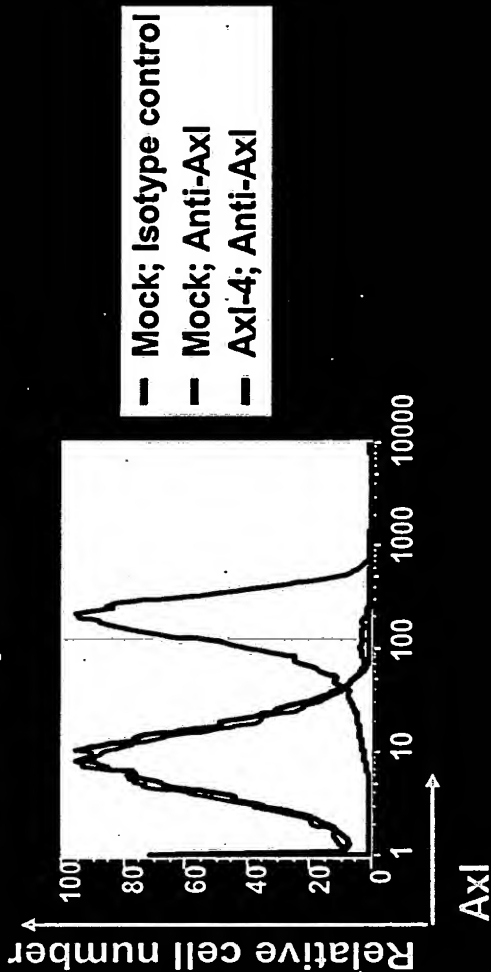


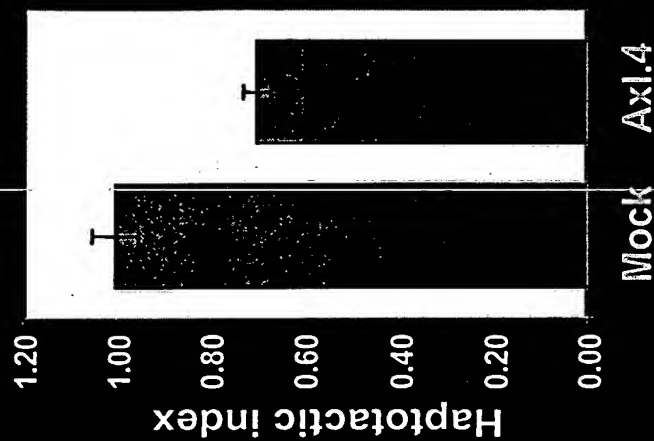
Figure 12

# Axl-4 RNAi Knocks Down Axl Protein Expression and Phenocopies Screening Hit Inhibition of VN Haptotaxis

FACs at 48 hrs post transfection



VN Haptotaxis at 48 hrs  
post transfection



Western Blot at 48 hrs post transfection

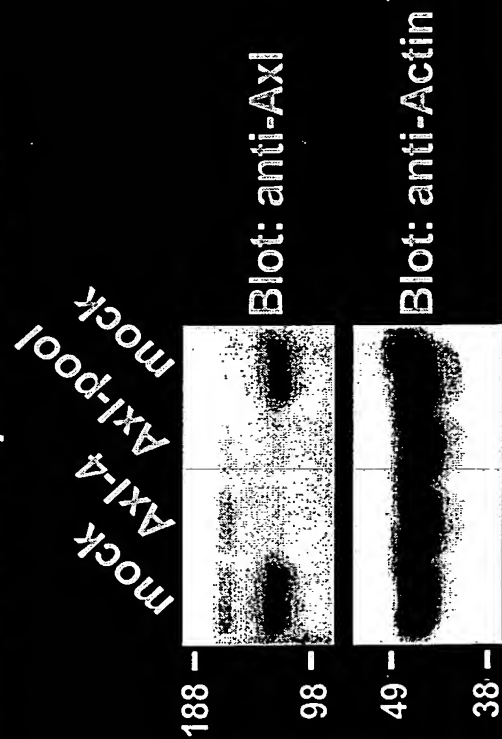
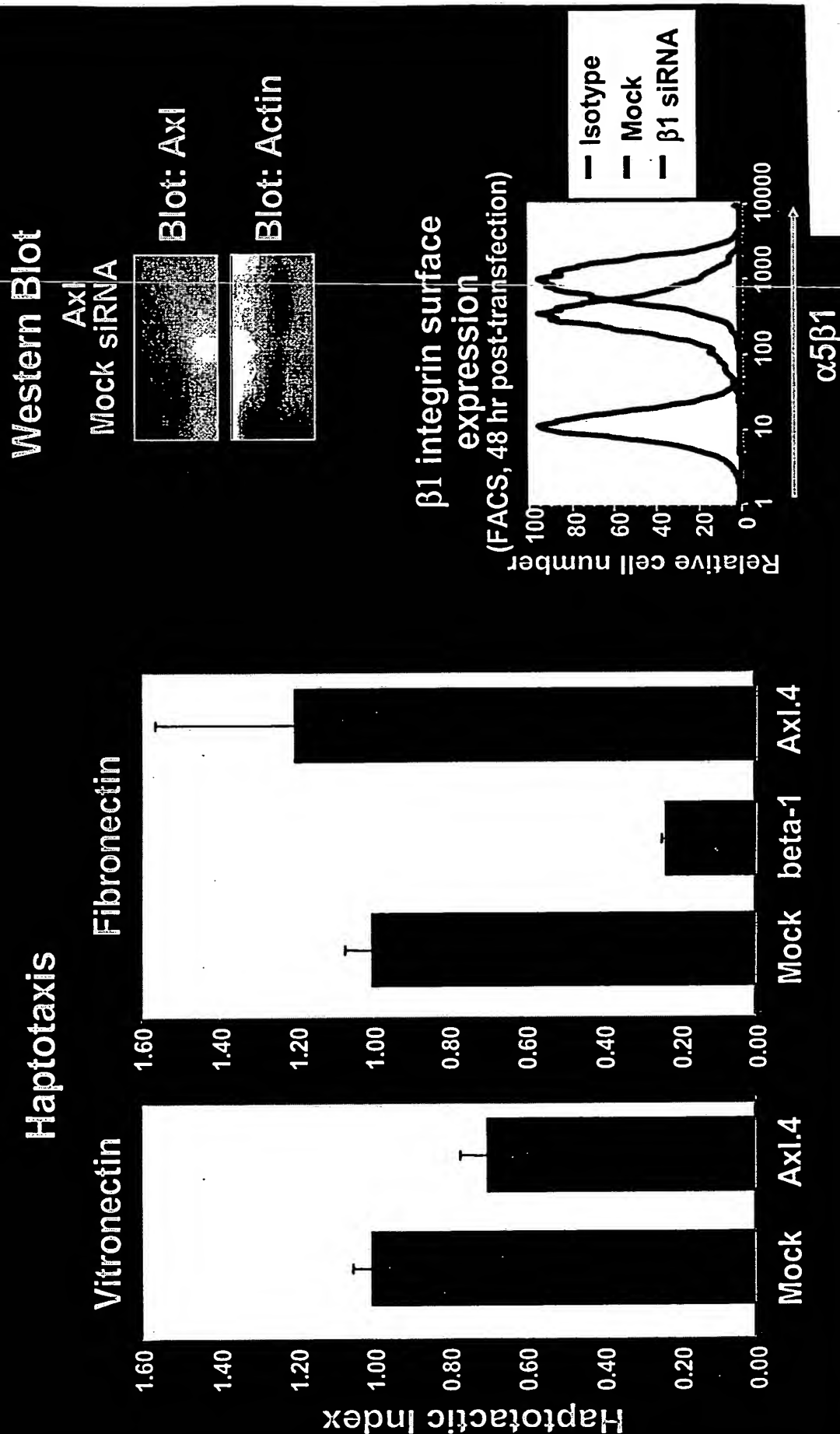


Figure 13

# Axl RNAi Inhibits Haptotaxis to Vitronectin not Fibronectin



三

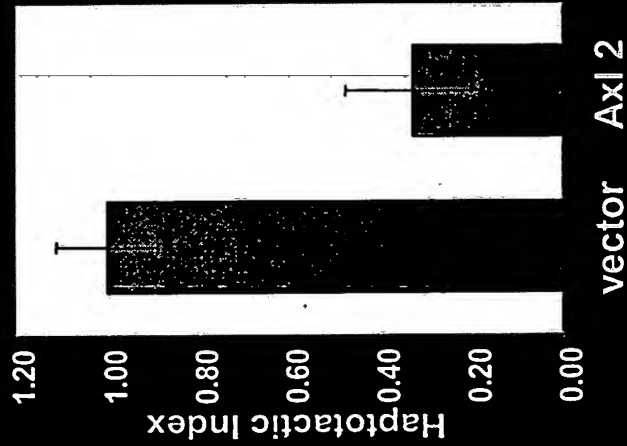


Figure 15

# Axl RNAi Vector Inhibits VN Haptotaxis and HUVEC Proliferation

Infect HUVEC with RNAi vector → Follow % GFP over several days

VN Haptotaxis



Proliferation (% GFP)

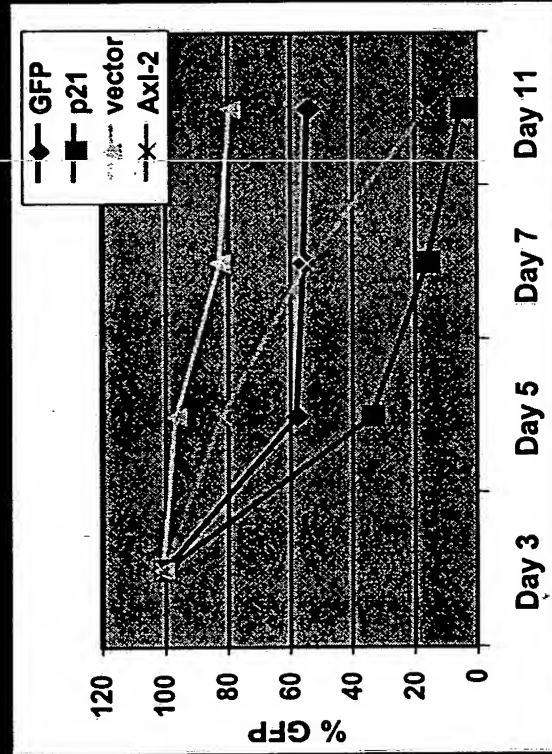




Figure 16

# Axl Extracellular Domain was Isolated In VEGFR2 Screen

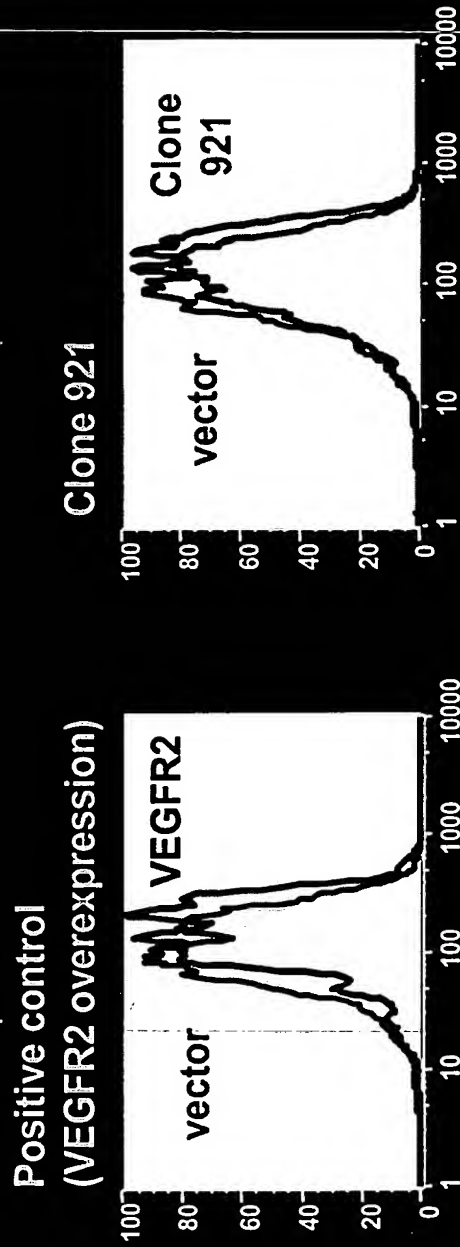
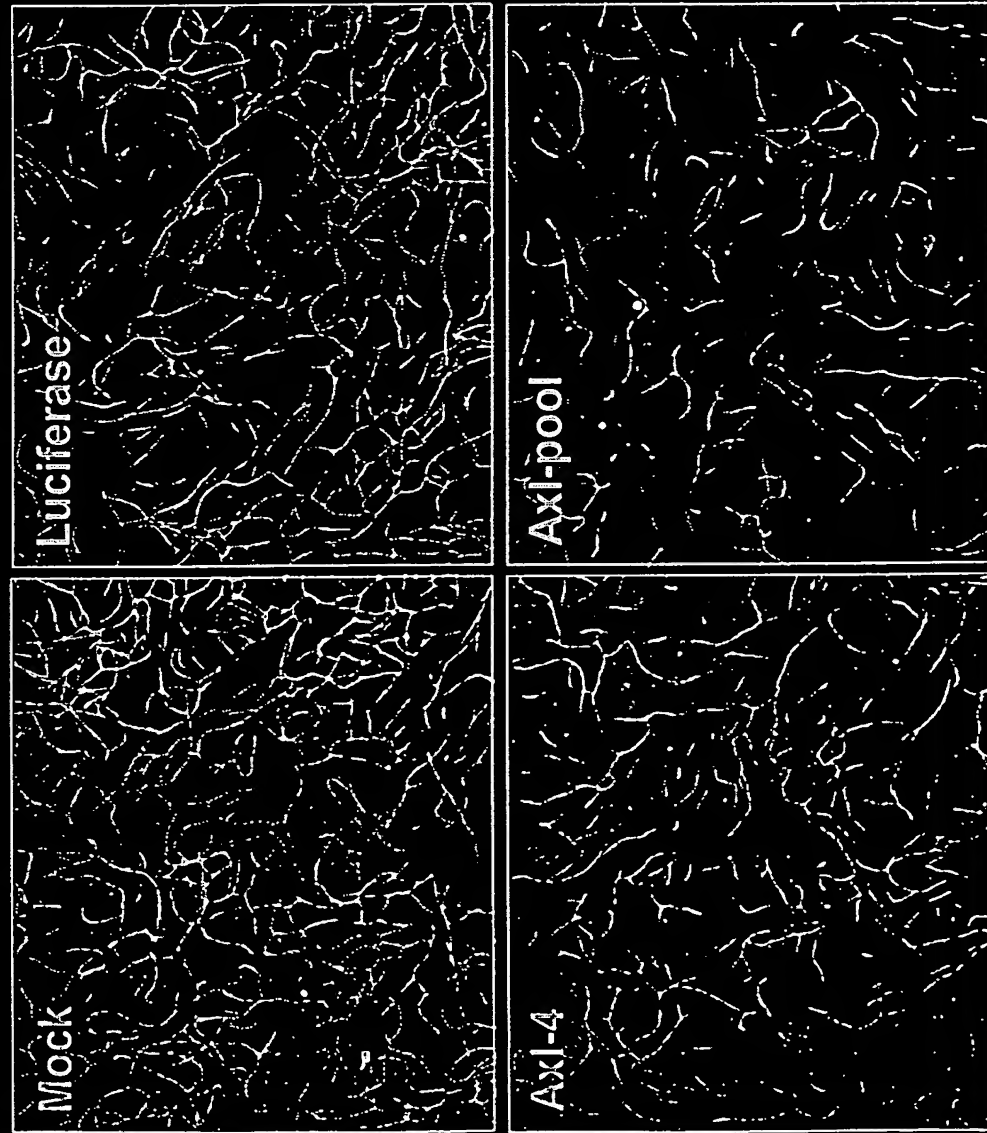




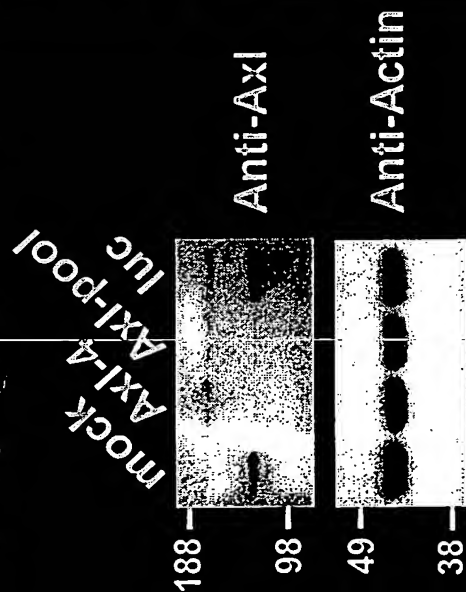
Figure 17

# Axl RNAis Inhibit Tube Formation in the Co-Culture Assay

Co-Culture of PAMCs with RNAi-transfected HUVEC  
Fixed at 5 days; stained with anti-PECAM-FITC



Western blot; whole cell lysate  
48 hrs post transfection



Quantified Mean Total Area of Tubules

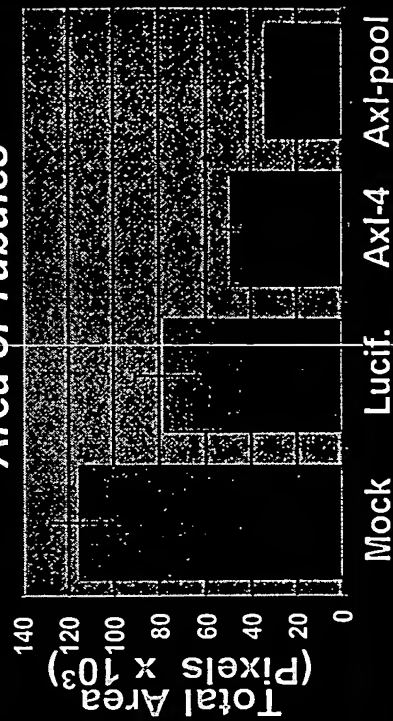
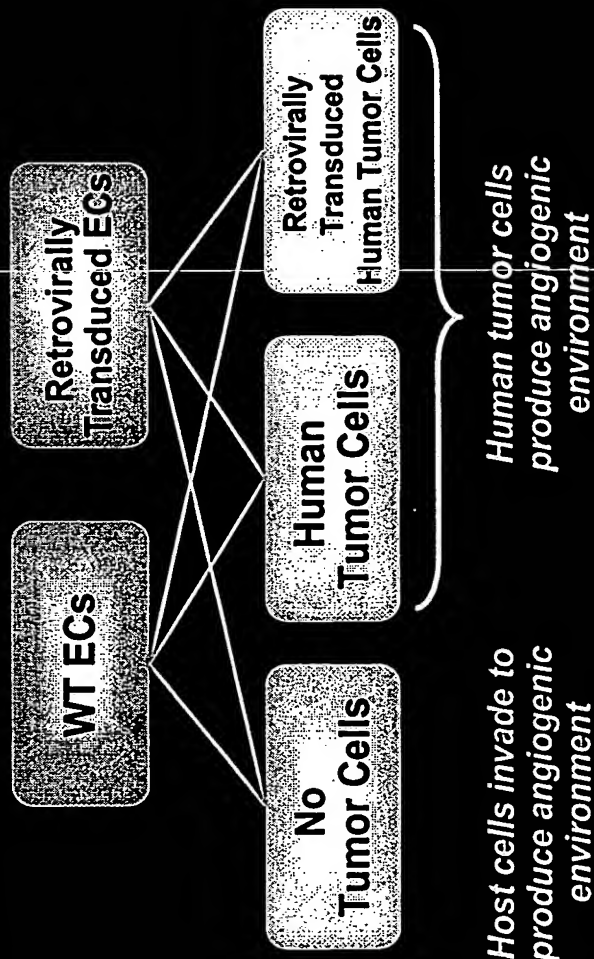
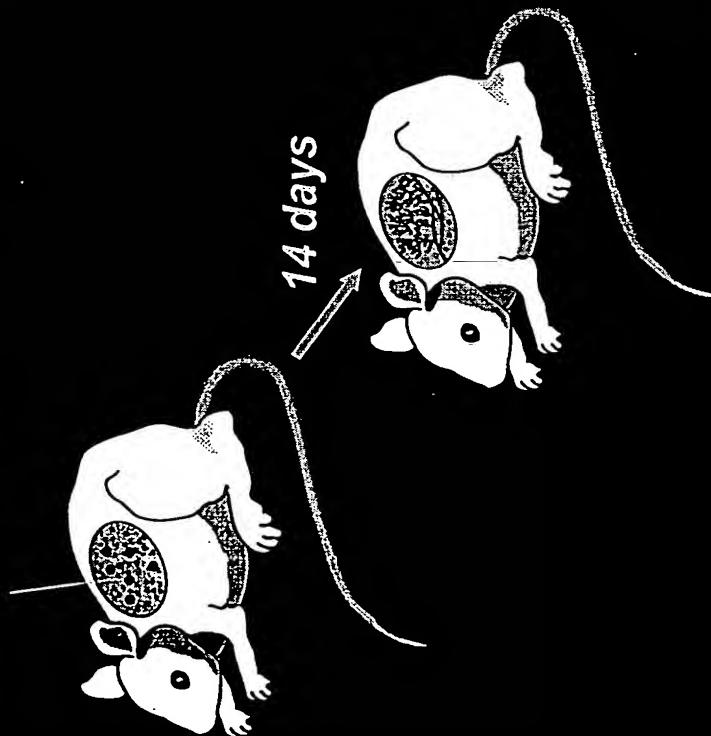


Figure 18

# Sponge Angiogenesis / Xenograft Model (Jaques Nör)



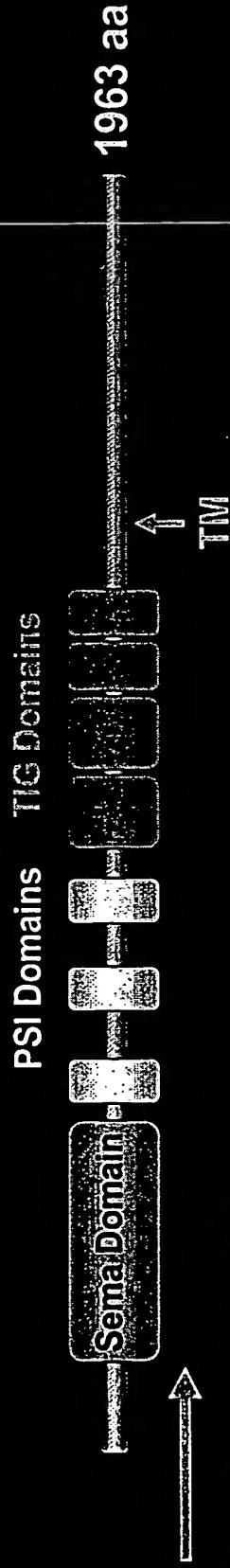
PLA "Sponge" with human ECs +/- human tumor cells



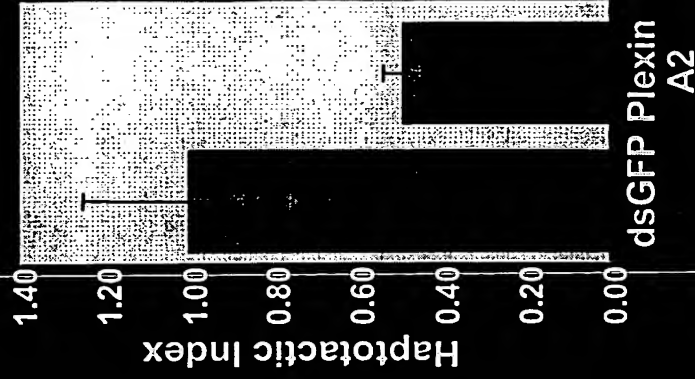
- Human ECs incorporated into a chimeric neovasculature.
- Can also monitor effects on human tumor cell growth
- Quantification of effect would entail determining the ratio of Human ECs to mouse ECs.
- Require an immune-suppressed mouse strain (SCID).

Figure 19

# Plexin-A2 (GH1-204)



GFP Screening Hit



- Sense hit
- Plexins are semaphorin co-receptors, but lack enzymatic domains
- Plexin A2 co-receptor for Sema3F ( $\alpha\text{v}\beta 3$  screen hit) which has been recently described as anti-angiogenic ligand
- Plexins associate with neuropilins via N-terminus
- Plexin A2 expression mainly restricted to endothelial cells and brain (SOURCE)

Figure 20

# Deoxycytidylate Deaminase (GH1-27)



GFP Screening Hit

- Sense hit
- Regulates pyrimidine deoxynucleotide metabolism
- Impairment affects fidelity of DNA replication
- Overexpressed in many malignant tumors and aggressive lymphoid malignancies
- Upregulated following myocardial infarction
- Inhibited by tetrahydrodeoxyuridine (H4U)
- Combination of H4U and 5-fluorodeoxycytidine (2'deoxyctidine kinase substrate) inhibits Lewis Lung carcinoma growth in mouse model

